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Remodeling of the tumor microenvironment predicts increased risk of cancer in postmenopausal women-The Prospective Epidemiologic Risk Factor (PERF I) Study

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Running title: C1M, C4M and VICM predicts increased risk of cancer

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Abstract

Background: An altered tumor microenvironment is one of the earliest signs of cancer and an important driver of the disease. We have seen previously that biomarkers reflecting tumor microenvironment modifications, such as, matrix metalloproteinase (MMP)-degraded type 1 collagen (C1M), MMP-degraded type IV collagen (C4M) and citrullinated and MMP-degraded vimentin (VICM), were higher in the serum of cancer patients than in healthy controls. However, it is not known if these biomarkers could predict an increased risk of cancer. The aim of this study was to investigate whether C1M, C4M and VICM were elevated prior to diagnosis of solid cancers in a large prospective study.

Material and Methods: Between 1999 and 2001, 5855 postmenopausal Danish women aged 48-89 enrolled in the Prospective Epidemiologic Risk Factor study. Baseline demographics and serum were collected at the time of registration. Follow up cancer diagnoses were obtained from the Danish Cancer Registry in 2014. Serum C1M, C4M and VICM levels were measured by competitive ELISAs.

Results: A total of 881 women were diagnosed with solid cancers after baseline. C1M, C4M and VICM levels were significantly elevated in women diagnosed less than 1 year after baseline. C1M and VICM, but not C4M, were independent predictors of increased risk of cancer.

Conclusion: C1M, C4M and VICM are elevated prior to cancer diagnosis. C1M and VICM are both independent predictors of increased cancer risk.

Impact: C1M and VICM are predictors for increased risk of cancer.

Introduction

In the US, the population aged 65 and older is expected to double by 2060 and the incidence of cancer is estimated to increase among the elderly by 67% (1,2). Cancer is therefore expected to remain one of the leading causes of death among 65-85 year olds in the Western world (3). Early detection of cancer greatly improves the chances of survival. There is therefore a need for early detection of cancers, especially in the elderly. This could be accomplished by developing non-invasive biomarkers for selection of high-risk subjects eligible to enter cancer screening programs.

One of the earliest signs of cancer is an altered tumor microenvironment which involves the appearance of cancer associated fibroblasts, recruitment of tumor-associated macrophages, enhanced extracellular matrix (ECM) deposition and protease secretion (4). Failure to recruit a permissive tumor microenvironment is believed to be the reason some tumors remain dormant and in accordance an altered and collagen-dense microenvironment is considered a potential risk factor for development of invasive cancers (5,6).

We recently developed non-invasive biomarkers that reflect modifications in the tumor microenvironment. These markers measure matrix metalloprotease (MMP)-degraded type I collagen (C1M), MMP-degraded type IV collagen (C4M) and citrullinated and MMP-degraded vimentin (VICM) (7–9).

C1M reflects interstitial matrix remodeling and measures a type I collagen degradation fragment generated by cleavage with MMP-2, -9 and -13 (8). Type I collagen is one of the most abundant interstitial ECM proteins. It is involved in maintaining tissue architecture and serves as a barrier for migration of epithelial cells under healthy conditions (10). During cancer progression however, type I collagen is often dysregulated and remodeled by MMPs (11). The architecture of type I collagen also changes. It stiffens and becomes cross-linked, which together with MMP-driven tissue degradation, drives cell invasion and migration (12).

C4M reflects basement membrane (BM) remodeling and invasion, and measures a type IV collagen α1-chain fragment generated by cleavage with MMP-12 (7). A hallmark of the malignant process is the

acquisition of an invasive phenotype that enables malignant cells to invade the BM (13). The BM is a compact specialized ECM structure that consists mainly of type IV collagen and laminin (14). Studies show that the cellular invasion through the BM in cancer is mainly driven by increased MMP activity which allows the malignant cells to enter the interstitial matrix and spread to distant sites (15,16).

VICM is believed to reflect intermediate filament (IF) remodeling and inflammation and measures a citrullinated fragment of vimentin generated by cleavage with MMP-2, -3, -8, -9, -12 and -13. Vimentin is used as a marker for epithelial-to-mesenchymal transition (EMT) and can be secreted from activated macrophages and endothelial cells into the microenvironment for further processing by MMPs (17–21). Furthermore, vimentin is known to undergo citrullination by protein arginine deiminases that are overexpressed in malignant tumors and associated with inflammation (18,22).

We have previously seen that C1M, C4M and VICM levels are elevated in serum of cancer patients compared with serum levels measured in healthy controls (21,23,24), but it is not known if these biomarkers are elevated prior to the diagnosis of cancer. The aim of this study was to investigate whether levels of C1M, C4M and VICM in serum samples collected in a large prospective study of postmenopausal women could be used as predictors of increased risk of cancer.

Material and Methods

Study design

The Prospective Epidemiologic Risk Factor (PERF I) study aims at finding risk factors associated with age-related diseases, as previously described by Dragsbæk et al (25). A total of 5855 Danish postmenopausal women aged 48-89 enrolled in the PERF I study during 1999-2001 (baseline). Follow up registry data from the Danish Cancer Registry were collected in 2014. Women who had previously either participated in clinical randomized placebo-controlled studies or had been screened for previous studies at the Center for

Clinical and Basic Research in Denmark were invited to participate in PERF I. The PERF I study was carried out in accordance with ICH-GCP and the study protocol was approved by the local ethics committees.

Selection of cases and controls

Registry data was collected in 2014 from the Danish Cancer Registry. The last updated data were entered in the registry in December 2012, leading to an average follow up time of 12.1 years of women enrolled in PERF I. Cancer subtypes were classified according to WHOs international Classification of Diseases 10 (ICD10), and reported in this paper as cancer of the breast (C50), digestive organs (C15-C26), respiratory organs (C30-C39), female genital organs (C51-58) and other organs (C40-C41, C43-C44, C45-C49, C64-C68, C69-C72, C73-C75, D00-D09).

Subjects diagnosed after baseline with a malignant neoplasm were included as cases (n=881). Excluded from the cases group were subjects with benign cancers (D10-D36), non-melanoma skin cancer (C44), hematological cancers (C81-C96), desmoplasia (N87) and neoplasms of uncertain behavior (D37-D48). If subjects had more than one cancer diagnosis after baseline the diagnosis closest to baseline was used in the analysis. Subjects with a cancer diagnosis solely prior to baseline were also excluded from the analysis (Figure 1).

Subjects with no history of cancer, and no connective tissue-, circulatory-, respiratory-, digestive-, musculoskeletal- and genitourinary system diseases (ICD10 chapter II, IX, X, XI, XIII, XIV) were included as controls and are referred to as healthy women (n=528) in this paper.

Baseline investigations

At baseline subjects reported on demographic characteristics together with current smoking status, alcohol consumption, physical activity and level of education as well as whether they were receiving treatment for hypertension or hyperlipidemia. The baseline characteristics of the PERF I subpopulation studied in this article is representative of the entire PERF I cohort ((25)). Vital signs and fasting serum samples were collected at time of enrollment and serum samples were stored at -80°C for later analysis.

Laboratory measurements

MMP-degraded type I collagen (C1M) (n=5629), MMP-degraded type IV collagen (C4M) (n=5630) and citrullinated and MMP-degraded vimentin (VICM) (n=5630) were measured blinded in serum by competitive enzyme-linked immunosorbent assays (ELISA) in a CAP-certified laboratory as described by Leeming et al (8), Sand et al (7) and Vassiliadis et al (9).

The serum samples were tested for stability and were considered to be stable after storage at -80°C. In detail, a three year stability study were performed, where the biomarker levels were measured in one year intervals. Furthermore, 10 freeze thaw cycles were done with no significant change in biomarker levels. Lastly, the median biomarker levels measured in this study was comparable to median biomarker levels measured in other similar studies with shorter storage times.

Serum samples were measured in double determinations and the coefficient of variation (CV) was <15%. The intra- and inter-assay variations were <10% and <15% respectively.

Statistical analysis

Baseline characteristics of women with cancer events and healthy women were compared using a Mann-Whitney test for numerical variables and a chi-square test for categorical variables.

The levels of C1M, C4M and VICM were compared using a Kruskal–Wallis test where the median biomarker levels of women diagnosed with cancer <1 year after baseline were compared with the biomarker levels of the healthy women, and with women diagnosed with cancer 1-2 years, 2-4 years and >4 years after baseline.

A Spearman correlation test was used to examine a possible relationship between days to cancer diagnosis after baseline and serum levels of C1M, C4M and VICM.

Univariable and multivariable logistic regression analysis were performed to assess the odds ratios (OR) and the area under the receiver operating characteristic curve (AUC) for the biomarkers and selected risk factors. Healthy women and women diagnosed with cancer <1 year after baseline were included in the analysis. C1M, C4M and VICM levels were analyzed on a continuous scale and split into two groups based on median levels (38.6 ng/ml for C1M, 71.7 ng/ml for C4M and 3.3 ng/ml for VICM). In the multivariable analysis a backward selection method was used to identify the best prediction model and to analyze the independent nature of the biomarkers. To correct for overfitting, an internal validation was conducted by calculating the bootstrap optimism-corrected AUC (The data was resampled 1000 times with the bootstrapping method*).* A covariate, defined as the time lag between the cancer diagnosis and baseline, was introduced into the model.

The statistical analyses were performed using MedCalc Statistical Software v.12 (MedCalc Software, Ostend, Belgium), R software (2.15.1 version, R Development Core Team, 2012) and GraphPad Prism v.6 (GraphPad Software, La Jolla, USA).

Results

Cohort Characteristics

From the PERF I study population two major groups were defined; women diagnosed with cancer after baseline (n=881) and healthy women with no history of cancer, and no ECM- or inflammatory- related diseases (n=528). The women diagnosed with cancer after baseline were further subdivided according to time of diagnosis and tumor stage (Figure 1).

Table 1 summarizes the baseline characteristics of the total cohort and the two groups. The median age of the total population at baseline was 71.3 years, and the median age of the women diagnosed with cancer after baseline was significantly higher than that of the healthy women (71.9 versus 70.2 years) ($p<0.0001$). The entire cohort was characterized by being slightly overweight (BMI 25.5) with the healthy women having a significantly lower BMI than women with cancer events (BMI 25.2 versus 25.8) ($p=0.0007$). Compared with the healthy women, the group with cancer had a significantly higher percentage of current smokers (26.2% versus 20.7%) ($p=0.02$), a significantly lower proportion of physically active subjects (71.8% versus 81.0%) ($p=0.0001$), a lower percentage educated to high school level (19.4 % versus 22.3%) (p=ns) and a non-significant higher proportion of alcohol-consumers drinking ≥ 7 drinks/week (34.5% versus 31.1%) (p=ns). The healthy women compared with the women with cancer had a significantly lower proportion women on hypertensive treatment (23.9% vs 33.1%) ($p=0.0003$) and hyperlipidemia treatment (8.7% vs 7.5%) (p=ns).

The serum C1M and C4M levels, but not VICM (3.5ng/ml vs 3.1 ng/ml), were significantly higher (41.1 ng/ml vs 38.0 ng/ml and 72.7 ng/ml vs 71.0 ng/ml) ($p = 0.0001$ and $p=0.02$) in women diagnosed with cancer after baseline compared with the healthy women. When age matching the cohort C1M and C4M levels continued to be significantly different in women diagnosed with cancer after baseline than healthy controls (data not shown).

C1M, C4M and VICM are significantly elevated 1 year prior to cancer diagnosis

Patients diagnosed with cancer after baseline were divided into 4 groups: women diagnosed <1, 1-2, 2-4 and >4 years after baseline. The levels of C1M, C4M and VICM were all elevated in serum from women diagnosed <1 year after baseline compared with women diagnosed >4 years after baseline and healthy women.

In detail, C1M levels were significantly elevated in serum from women diagnosed <1 year after baseline compared with women diagnosed >4 years after baseline and healthy women (p=0.019 and p=<0.0001, respectively) (Figure 2A). C4M levels were significantly elevated in serum from women diagnosed <1 year after baseline compared with women diagnosed 1-2, 2-4, >4 years after baseline and healthy women (p=0.02, p=0.03, p=0.04 and p=0.002, respectively) (Figure 2B). VICM levels were significantly elevated in serum from women diagnosed <1 year after baseline compared with women diagnosed >4 years after baseline and healthy women (p=0.0075 and p=0.0071, respectively) (Figure 2C). When the biomarker levels in women diagnosed with cancer <1 year after baseline were compared with the levels of all women without a cancer diagnosis (n=4015), C1M, C4M and VICM continued to be significant (data not shown).

C1M, C4M and VICM levels all correlated to time of cancer diagnosis up to 1000 days after baseline (r= -0.14 ,p=0.047; r= -0.17, p=0.011 and r= -0.18, p=0.0067, respectively) (Supplementary figure S1A, S1B and S1C). The significant p value may however be due to the large power in this study and not necessarily reflect a real correlation between days and biomarker levels.

Together, these findings indicate that changes in C1M, C4M and VICM could be useful for prediagnostic risk assessments as elevated levels of the biomarkers indicate risk of a cancer diagnosis within a year.

C1M, C4M and VICM levels are associated with cancer stage

The women diagnosed with cancer up to 1 year after baseline were divided into 3 groups: women diagnosed with localized cancers, lymphovascular invasions or metastases. The median C1M, C4M and VICM levels were highest in serum from women diagnosed with metastasized cancers. C1M levels were significantly elevated in patients diagnosed with metastasis compared to healthy controls (p=0.01) (Figure 3A). C4M levels were significantly elevated in patients diagnosed with metastasis compared with subjects diagnosed with localized cancers and healthy controls (p= 0.002 and p=0.03) (Figure 3B). Lastly, VICM levels were significantly elevated in patients diagnosed with metastasis compared with healthy controls (p=0.05) (Figure 3C).

C1M and VICM independently predict an increased risk of cancer

Univariable logistic regression was used to assess C1M, C4M, VICM levels as well as risk factors of cancer development (age, smoking, BMI, physical inactivity, alcohol consumption, education level, hypertension, and hyperlipidemia) in women diagnosed with cancer up to 1 year after baseline compared with healthy women. Biomarkers were analyzed on a continuous scale as well as split into two groups based on median levels.

C1M, C4M and VICM, age, smoking, exercise and hypertension were all individual predictors of cancer up to 1 year prior to diagnosis. In detail, women with high levels of C1M and VICM were 2.2 and 2.3 times more likely to develop cancer within the first year after blood draw than women with low levels of C1M and VICM (OR=2.2, AUC=0.60, p=0.0006, OR=2.3, AUC=0.60, p=0.0003, respectively). Women with high levels of C4M were 1.6 times more likely to develop cancer within the first year after blood draw than women with low levels of C4M (OR=1.6, AUC=0.57, p=0.031) (Table 2).

To test if C1M, C4M and VICM were independent predictors of cancer, a multivariable logistic regression model was made. A backwards selection of the markers yielded in C1M and VICM levels, age, smoking and exercise being statically significant, and the bootstrapped AUC was 0.70. This suggests that both C1M and

VICM, but not C4M, levels up to 1 year prior to diagnosis were independent predictors of cancer in this cohort of elderly women (Table 3). The statistically significant association between C1M, VICM and cancer diagnosis was slightly attenuated (OR=1.8 vs. OR=2.2 for C1M and OR=1.9 vs. OR=2.3 for VICM) when the markers were included in the multivariable logistic regression model.

Discussion

In the current study we showed that C1M and VICM, but not C4M, are independent predictors of increased risk of cancer in postmenopausal women. In detail, we found that postmenopausal women with high levels of C1M, C4M and VICM were more likely to develop cancer within the first year after baseline than women with low levels of the biomarkers. Furthermore, we found that women had significantly increased C1M, C4M and VICM levels up to 1 year prior to cancer diagnosis.

These findings correspond well with the literature where studies show that vimentin, increased MMP expression, basement membrane degradation and increased collagen density are markers of poor prognosis (6,18,26,27). Studies showed that vimentin is over-expressed in majority of cancers and is generally associated with a metastatic phenotype and poor prognosis (18). Similarly, MMP-2 and MMP-9 are often found elevated in tumor tissues (9,28) and MMP-mediated collagen degradation has been shown to be predictive of cancer mortality in studies in elderly women (25,29). This indicates that subjects with high levels of collagen remodeling have increased chances of both developing and dying from cancer.

It is possible that increased circulating C1M and C4M levels mirror a more active ECM remodeling response. If a cancer spreads and invades distant tissues, it encounters two kinds of ECM barriers: the dense endothelial basement membrane and the underlying more porous interstitial matrix. It is likely that C4M mirrors basement membrane remodeling and C1M, interstitial matrix remodeling. During cancer progression, both ECM barriers are remodeled resulting in a loss of epithelial polarity and uncontrolled growth (30,31).

Similarly, it is possible that increased circulating VICM levels mirror a more active inflammatory response. During cancer progression innate immune cells are recruited to the tumor microenvironment where they contribute to cancer development, mostly due to their secretion of inflammatory mediators that regulate proliferation, angiogenesis and tissue remodeling (14,32,33)

Results suggest that inflammatory conditions and ECM remodeling are present before a malignant change occurs (34,35). Whether inflammation and ECM remodeling are sufficient for the development of cancer is still unclear. However, it is well accepted that modification of the tumor microenvironment is one of the earliest signs of cancer. This could explain why high C1M, C4M and VICM serum levels were observed prior to diagnosis of cancer in this study cohort.

There are several limitations to this study. We saw that the biomarker levels increased with tumor stage. It therefore seems likely that the markers are secreted directly from the tumor tissue. The number of subjects in each category is low and from this study we cannot establish causality. It is possible that the high levels of C1M, C4M and VICM in the blood are results of a systemic effect where subjects with a general increased remodeling of the ECM and IF have a higher likelihood of developing cancer. Furthermore, all markers have been found elevated in a number of diseases characterized by ECM remodeling and tissue inflammation and are therefore not specific to cancer (7–9,36–39). Lastly, it needs to be elucidated if the results of this study can be applied to populations of other ages and ethnic backgrounds and if the biomarkers are more useful for certain types of solid cancer.

Despite these limitations, C1M and VICM may play a future role as predictors of cancer risk in combination with age, exercise and smoking status. There is a great need to identify predictors of cancer risk for implementation of cancer screening programs. Screening entire populations is expensive and may cause patients unnecessary worry or harm, such as from over-diagnosis, false-positives and radiation risk (40,41). For successful implementation of national screening programs it is therefore necessary to identify high risk subjects to maximize the harm-benefit and cost-effectiveness ratios (40,41). Additionally, currently

screening trials favor younger adults, but as life expectancy increases it will become more important to include elderly subjects in screening and clinical trials (1).

In conclusion, we saw that C1M, C4M and VICM, were elevated up to 1 year prior to cancer diagnosis. C1M and VICM were both independent predictors of increased risk of cancer in this study of postmenopausal Danish women. Measurement of modification in the tumor microenvironment could be useful for prediction of increased risk of cancer.

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Table 1 Patient Characteristics

Cohort Characteristics	Total cohort n=1410		Women with cancer dx n=881		Healthy women * n=528		p-value
	%	No.	%	No.	%	No.	
Age at time of BD, years							
Median (No.)	71.3 (n=1410)		71.9 (n=881)		70.2 (n=528)		<0.0001
95% CI	70.9-71-6		71.4-72.6		69.1-70.9		
BMI							
Median (No.)	25.5 (n=1354)		25.8 (n=844)		25.2 (n=509)		0.0007
95% CI	25.3-25.7		25.4-26.2		24.9-25.5		
Current Smoking (yes/no)	24.2	339/1403	26.2	231/881	20.7	108/521	0.02
Alcohol (≥7 drinks/week)	33.3	464/1394	34.5	302/876	31.1	161/517	0.24
Current exercise (≥1 h/week)	75.3	1055/1402	71.8	632/880	81.0	422/521	0.0001
Education:							
Primary school	72.0	1007/1402	73.4	646/880	69.1	360/521	0.22
High school	20.2	287/1402	19.4	171/880	22.3	116/521	
University	7.8	108/1402	7.2	63/880	8.6	46/521	
Hypertension treatment	29.5	439/1487	33.1	291/880	23.9	124/520	0.0003
Hyperlipidemia treatment	8.1	121/1489	8.7	77/881	7.5	39/521	0.46
Serum C1M, ng/ml							
Median (No.)	39.5 (n=1349)		41.1 (n=844)		38.0 (n=505)		0.0001
95% CI	38.6-40.7		39.5-42.9		36.5-38.9		
Serum C4M, ng/ml							
Median (No.)	71.8 (n=1350)		72.7 (n=845)		71.0 (n=505)		0.02
95% CI	70.8-73.1		71.1-74.2		68.5-72.8		
Serum VICM, ng/ml							
Median (No.)	3.4 (n=1350)		3.5 (n=845)		3.1 (n=505)		0.17
95% CI	3.2-3.5		3.3-3.7		3.0-3.4		
Years to cancer diagnosis:							
<1 year			12.0	106/881			
1-2 years			7.7	68/881			
2-4 years			17.3	152/881			
>4 years			63.0	555/881			
Cancer type:							
Breast			22.6	199/881			
Digestive organs			22.7	200/881			
Respiratory organs			11.8	104/881			
Female genital organs			10.3	93/881			
Other			19.4	171/881			
Unknown			12.9	114/881			

Abbreviations: BD, Blood Draw; BMI, Body Mass Index; dx, diagnosis; h, hours; C1M, MMP-degraded type I collagen; C4M, MMP-degraded type IV collagen; VICM, citrullinated and MMP-degraded vimentin.

* Women with no history of cancer or diseases of connective tissue, circulatory-, respiratory-, digestive-, musculoskeletal- or genitourinary systems.

Table 2. Univariable analysis: women diagnosed with cancer within 1 year vs. healthy women

	AUC	95% CI	OR	95% CI	P-value
Serum C1M					
>38.6 ng/ml	0.596	0.564 to 0.640	2.203	1.406 to 3.452	0.0006
Continuous	0.629	0.589 to 0.668	1.007	1.003 to 1.011	0.001
Serum C4M					
>71.7 ng/ml	0.569	0.519 to 0.599	1.617	1.045 to 2.502	0.031
Continuous	0.609	0.569 to 0.648	1.013	1.005 to 1.022	0.002
Serum VICM					
>3.3 ng/ml	0.600	0.560 to 0.640	2.309	1.465 to 3.640	0.0003
Continuous	0.596	0.555 to 0.635	1.041	1.005 to 1.078	0.02
Age	0.628	0.589 to 0.666	1.080	1.042 to 1.118	<0.0001
BMI (≥ 25)	0.516	0.476 to 0.556	0.878	0.576 to 1.339	0.55
Current Smoking	0.561	0.522 to 0.601	1.885	1.194 to 2.977	0.007
Alcohol (≥7 drinks/week)	0.540	0.499 to 0.579	1.417	0.918 to 2.186	0.12
Exercise (≥1 time/week)	0.584	0.545 to 0.623	0.420	0.267 to 0.661	0.0002
Education:					
Primary school			1.00 (Ref)		-
High school	0.549	0.509 to 0.588	0.719	0.419 to 1.235	0.23
University			0.390	0.177 to 1.116	0.08
Hypertension treatment	0.565	0.525 to 0.604	1.859	1.193 to 2.896	0.006
Hyperlipidemia treatment	0.504	0.465 to 0.544	0.874	0.380 to 2.010	0.75

Table 3. Multivariable analysis: women diagnosed with cancer 1 year vs. healthy women

	AUC Adjusted	95% CI	OR	95% CI	P-value
Serum C1M (≥ 38.6 ng/ml)			1.7663	1.094 to 2.853	0.02
Serum VICM (≥ 3.3 ng/ml)			1.9646	1.210 to 3.191	0.006
Age	0.701	0.620 to 0.775	1.0718	1.031 to 1.114	0.0004
Exercise (≥1 time/week)			0.4436	0.268 to 0.735	0.002
Current Smoking			1.9192	1.163 to 3.168	0.01

Figure legends

Figure 1. Study flow diagram.

* Benign cancers, non-melanoma skin cancers, haematological cancers, desmoplasia, and neoplasms of uncertain behavior.

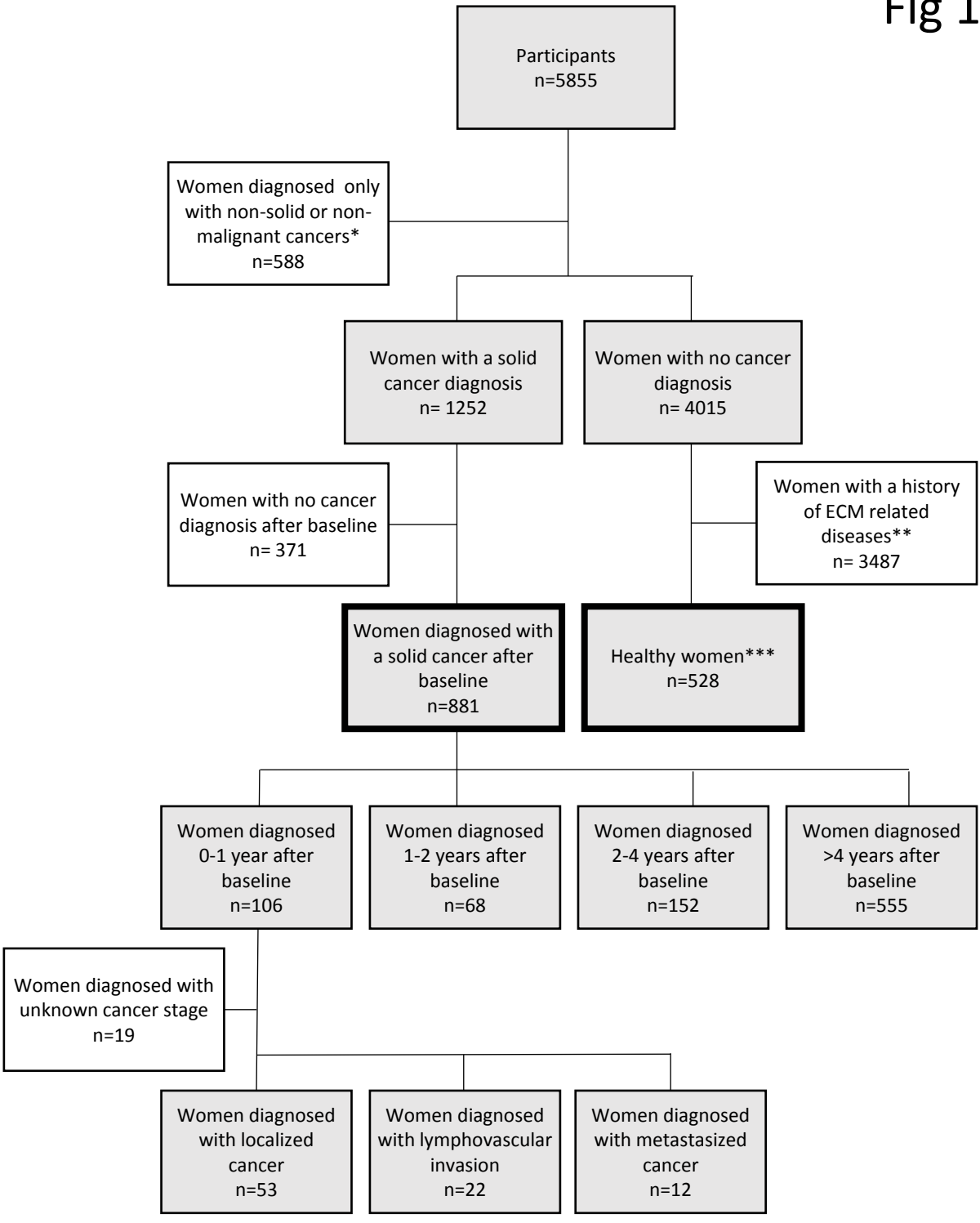
** Diseases of connective tissue, circulatory-, respiratory-, digestive-, musculoskeletal- and genitourinary systems.

*** Women with no history of cancer or diseases of connective tissue, circulatory-, respiratory-, digestive-, musculoskeletal- and genitourinary systems.

Figure 2. A, B, C) Prediagnostic distributions of serum biomarker levels. The boxes represent the 25th, 50th and 75th percentiles. The whiskers represent the lowest and highest value, except outliers (•), which are higher than 1.5 times the 75th percentile or lower than 1.5 times the 25th percentile. Groups were compared using a Kruskal Wallis test. Asterisks indicate the following: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ and ****: $p<0.0001$.

Figure 3. C1M (A), C4M (B) and VICM (C) serum levels in relation to cancer stage. The boxes represent the 25th, 50th and 75th percentiles. The whiskers represent the lowest and highest value, except outliers (•), which are higher than 1.5 times the 75th percentile or lower than 1.5 times the 25th percentile.

Fig 1



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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Fig 2

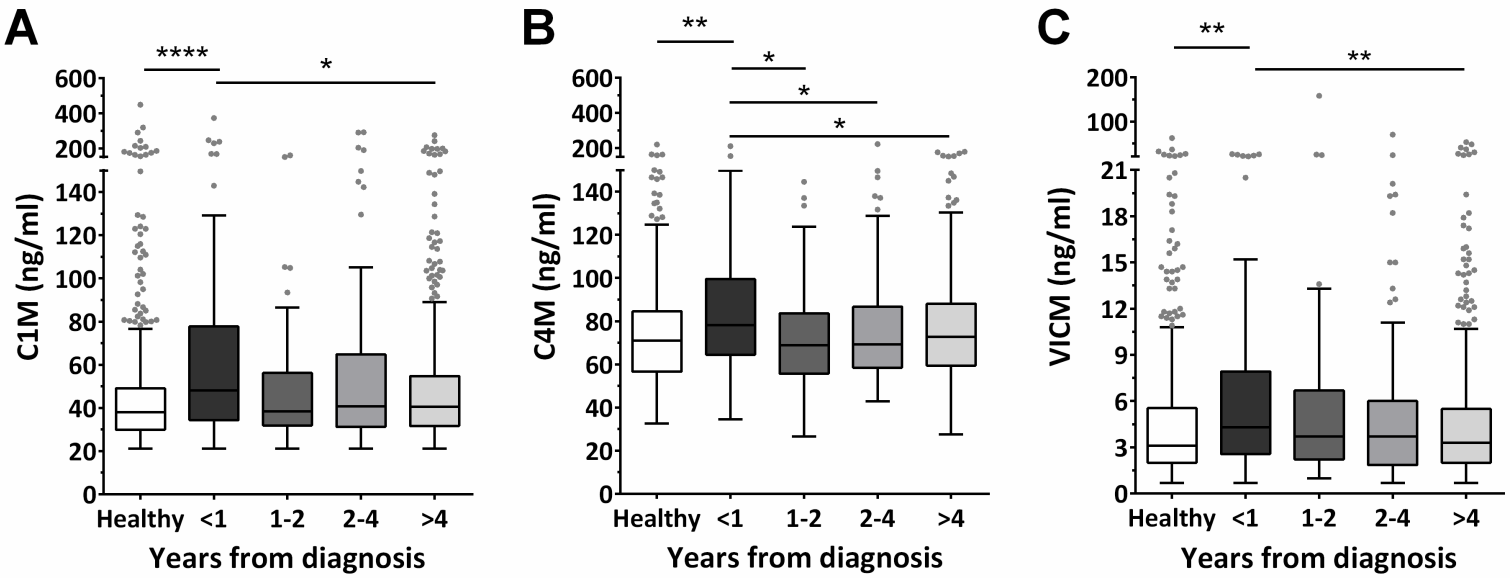

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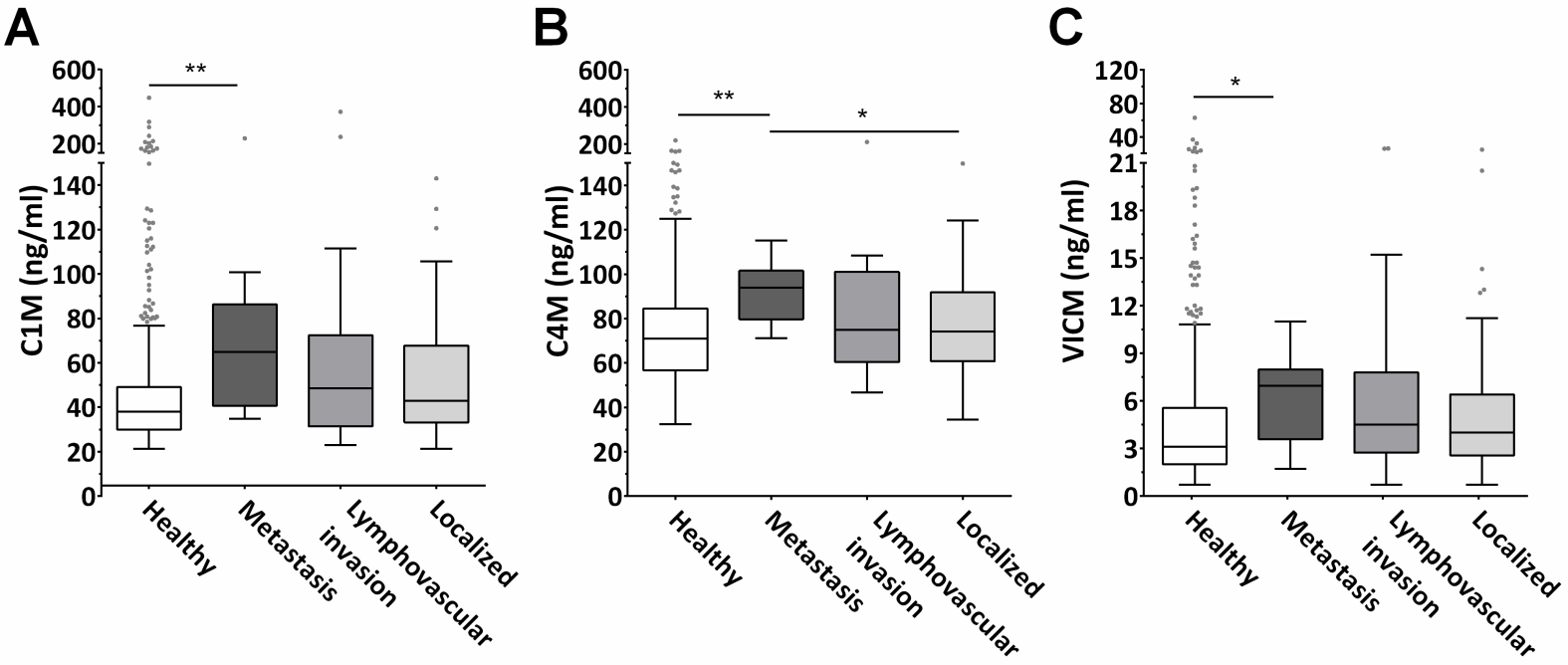
Fig 3



Cancer Epidemiology, Biomarkers & Prevention

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Cecilie Liv Bager, Nicholas Willumsen, Stephanie Nina Kehlet, et al.

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